Modulation of Intracellular Ceramide using Polymeric Nanoparticles to Overcome Multidrug Resistance in Tumor Cells

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Statement of Purpose: The development of multidrug resistance (MDR) in many tumor types is a major barrier to successful anti-cancer therapy, particularly in the treatment of breast and ovarian cancer where MDR develops in at least half of the clinical cases. Although MDR is known to develop through modulation of several molecular mechanisms within the tumor cell, the modulation of apoptotic signaling by the drug resistant tumor cell has been of considerable interest. Several MDR cancer cell types exhibit elevated levels of the enzyme glucosylceramide synthase (GCS), which is responsible for the bioactivation of ceramide, a messenger in pro-apoptotic signaling, to its non-functional moiety glucosylceramide. Inhibition of the apoptotic signal in MDR cells contributes greatly to the chemoresistance observed in these tumor types. The purpose of this study was to investigate the therapeutic strategy of co-administering C6-ceramide with paclitaxel, a commonly used chemotherapeutic, to potentially overcome MDR in a human ovarian tumor cell line (SK-OV3/TR). Moreover, the therapeutics were encapsulated into poly(ethylene oxide)-modified poly(ε-caprolactone)-(PEO-PCL) nanoparticles to enhance drug delivery to the tumor site specifically, thus enhancing overall efficacy of the drug therapy.

Methods: Drug-loaded PEO-PCL nanoparticles were prepared by controlled solvent displacement. Wildtype (drug sensitive) SK-OV3 ovarian cancer cells and the subculture of multidrug resistant SK-OV3/TR cells were cultured and maintained separately, where SKOV3/TR cells were specifically selected for paclitaxel resistance by supplementing the culture medium with 0.2 μM paclitaxel. Cells were plated in 96 well plates at 5000 cells/well for efficacy studies, and subjected to treatment with drug concentrations ranging from 0.1 nM to 10 μM for paclitaxel and 1 μM to 1 mM for ceramide, formulated for delivery in solution or in PEO-PCL nanoparticles, alongside treatments with appropriate vehicle controls. Treatment proceeded for 6 days after which cell viability was determined and quantified by MTS assay. In a separate set of experiments, cells were subjected to the same treatments, and fluorescence-stained for apoptotic activity 24 hours after initiation of treatment with a commercially available apoptosis assay kit. Apoptotic activity was quantified by cytometry and confirmed by fluorescence microscopy. In a third set of experiments, enhanced intracellular delivery of therapeutics with the nanoparticle formulation was visualized by loading the nanoparticles with rhodamine-paclitaxel and NBD-ceramide and, by fluorescent microscopy, monitoring drug delivery to the SK-OV3 and SK-OV3/TR cells at 0.5, 1, 2, and 6 hours of incubation.

Results and Discussion: Treatment of the multidrug resistant SK-OV3/TR cells with a 1 μM paclitaxel dose resulted in 65.65 ± 2.16% viability, while SK-OV3 cells showed 16.37 ± 0.41 % viability at a 100 nM dose, 10-fold lower than their drug resistant counterparts. Co-treatment of the MDR cells with 20 μM C6-ceramide in addition to 1 μM taxol resulted in a significant increase in cell death (2.69 ± 0.51% viability) compared with the taxol treatment alone ($p<0.001$). Similarly, addition of 10 μM C6-ceramide with the 100 nM paclitaxel dose to the wildtype SK-OV3 cells also resulted in enhanced cell death (7.38 ± 1.25% viability, $p<0.001$). Although the purpose of drug encapsulation within nanoparticles is for the *in-vivo* benefits of prolonged circulation and tumor-specific drug accumulation, delivery of the therapeutics encapsulated in PEO-PCL nanoparticles further enhanced the cell-kill response of paclitaxel and ceramide in drug resistant cells. A 10 nM dose of paclitaxel, delivered in combination with ceramide in PEO-PCL nanoparticles, resulted in 63.98 ± 4.90% viability, while delivery of the therapeutics as free drugs in solution at these doses did not provoke cell-kill in the MDR cells (110.58 ± 3.84% viability). This cytotoxicity profile shows that nanoparticle delivery of the co-therapy enhances chemosensitivity of the MDR cells to paclitaxel 100-fold, to produce a chemosensitivity profile in the MDR cells that is similar to their drug-sensitive counterparts. Visualization of intracellular nanoparticle delivery indicated that the nanoparticles are engulfed into the cell and translocated to the perinuclear region of the cell to deposit their drug load. This behavior may explain the enhanced chemosensitivity seen with nanoparticle delivery of the co-therapeutics to the MDR cells, since intracellular depot of the drug load bypasses P-glycoprotein efflux pumps that are expressed on the surface of the MDR cells. Apoptotic activity was quantified in response to treatment with either paclitaxel alone or in combination with ceramide, and it was found that addition of ceramide increased apoptotic activity 2-3 fold in the MDR cells, suggesting that delivery of exogenous ceramide reinstates the apoptotic signal to resensitize MDR cells to chemotherapy.

Conclusions: Altogether, co-administering paclitaxel with ceramide, delivered in polymeric nanoparticles appears to greatly re-sensitize drug resistant ovarian tumor cells to chemotherapy. The results demonstrate the great potential for clinical use of this therapeutic strategy to overcome MDR.

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